Antimicrobial peptide-loaded hyaluronic acid nanogels as a new strategy for tuberculosis therapeutics

Tuberculosis (TB), a disease caused by the human pathogen *Mycobacterium tuberculosis*, is one of the most deadly infectious diseases, along with HIV/AIDS. According to the most recent data, 9.6 million new cases of TB were diagnosed worldwide in 2014, 1.5 million of which resulted in the death of the patients.

Current treatments rely on expensive, long-lasting (from 6 to 24 months), multiple antibiotic therapies, being often associated with low patient compliance and usually resulting in treatment failure and emergence of multi-drug resistant mycobacterial strains. To top it off, Bacille Calmette-Guérin (BCG), the only vaccine available, fails in preventing TB in adulthood.

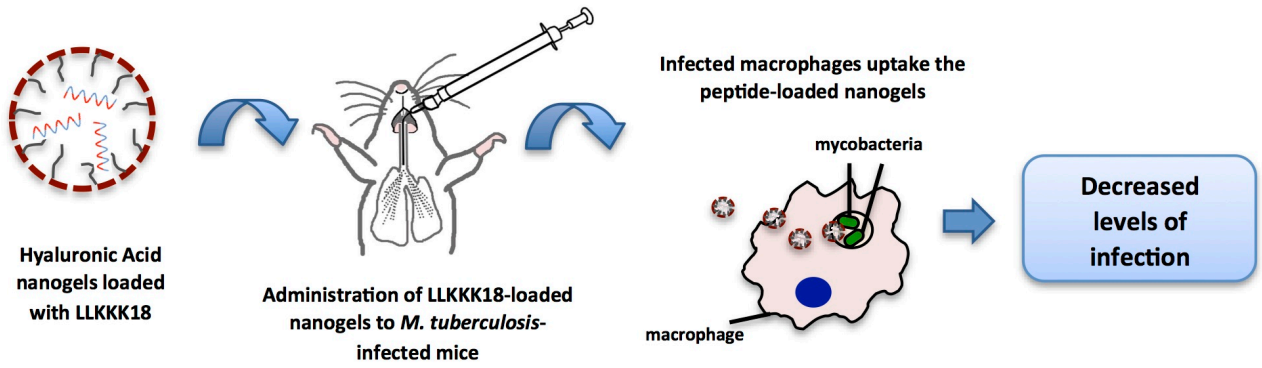

Fig. 1.

Antimicrobial Peptides (AMPs) comprise a group of small, cationic and amphipathic peptides that play a key role in the innate immune system of many living organisms. Their non-specific killing mechanism renders them a broader antimicrobial spectrum (compared to antibiotics) and reduced chances of mycobacteria acquiring resistance against them. For these reasons, AMPs arise as promising candidates for the treatment of TB. In particular, the ability of LL37 - the only known human cathelicidin (a family of AMPs) – to kill mycobacteria residing within the host cells has already been reported. Several analogues of LL37 have already been engineered to further improve its therapeutical potential. One such example is LLKKK18, whose modifications to the amino acid chain produced a smaller (only 18 amino acids long), more cationic and more hydrophobic peptide than LL37, ultimately showing higher antimicrobial activity.

A team comprising members from three different research centers – Center of Biological Engineering (CEB, University of Minho, Portugal), Life and Health Sciences Research Institute (ICVS, University of Minho, Portugal) and University of Porto (Porto, Portugal) – has recently

developed a promising strategy to treat tuberculosis, based on the exogenous administration of LLKKK18. This peptide was loaded into a self-assembling Hyaluronic Acid (HA) nanogel to increase LLKKK18's stability and prevent its degradability and cytotoxicity, while enhancing the targeting of the peptide to the main sites of infection.

Peptide-loaded nanogels were clearly internalized by infected macrophages and the peptide effectively co-localized with mycobacteria. This ultimately resulted in a significant reduction of the mycobacteria levels present in macrophages infected *in vitro* with either *Mycobacterium avium* (an opportunistic strain) or the human pathogen *M. tuberculosis*. Such effect was accompanied by a decrease in the concentrations of pro-inflammatory cytokines, supporting the reduced infection levels.

Most important, the LLKKK18-loaded nanogels were further administered intra-tracheally (through the use of an aerosolizer) to mice previously infected with *M. tuberculosis* and at a chronic stage of infection. Remarkably, only 5 or 10 every other day administrations significantly reduced the mycobacterial load in the lungs of infected mice.

Overall, the data obtained point towards a high potential of LLKKK18-loaded nanogels for the treatment of TB. It should also be noted that, compared to standard treatments, the use of an AMP is expected to: 1) encompass a lower risk of resistance acquisition; and 2) reduce therapy length, thus decreasing overall therapy costs. Nevertheless, additional studies still have to be carried out to further enhance the peptide's effect.

João Pedro Silva
CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal

Publication

Delivery of LLKKK18 loaded into self-assembling hyaluronic acid nanogel for tuberculosis treatment.
Silva JP, Gonçalves C, Costa C, Sousa J, Silva-Gomes R, Castro AG, Pedrosa J, Appelberg R, Gama FM
J Control Release. 2016 Aug 1